

**APPENDIX****WHAT IS CLAIMED:**

Claims 1-58. (Cancelled)

5 59. (Presently Amended) A method for identifying a ligand that binds to a protein, comprising the steps of:

(1) receiving unfolding data that indicates thermal unfolding as a function of temperature for a protein incubated with a molecule tested for binding;

(2) determining an unfolding temperature midpoint for the protein in the presence of the molecule from the unfolding data;

10 (3) comparing the unfolding temperature midpoint for the protein incubated with the ligand with the unfolding temperature midpoint for the protein in the absence of any molecules tested for binding; and

15 (4) determining that the molecule tested for binding binds to the protein when a difference between the unfolding temperature midpoint for the protein in the presence of the molecule and unfolding temperature midpoint for the protein in the absence of any molecules tested for binding exceeds a threshold; and

(5) estimating the ligand binding affinity at the unfolding temperature midpoint according to:

$$K_L^{T_m} = \frac{\exp \left\{ -\frac{\Delta H_u^{T_0}}{R} \left[ \frac{1}{T_m} - \frac{1}{T_0} \right] + \frac{\Delta C_{pu}}{R} \left[ \ln \left( \frac{T_m}{T_0} \right) + \frac{T_0}{T_m} - 1 \right] \right\}}{[L_{T_m}]}$$

where,

$K_L^{T_m}$  is a ligand association constant at  $T_m$ ;

$T_m$  is a midpoint temperature for the protein unfolding transition in the presence of the ligand;

$T_0$  is a midpoint temperature for the protein unfolding transition in the absence of the ligand;

$\Delta H_u^{T_0}$  is an enthalpy of protein unfolding in the absence of ligand at  $T_0$ ;

$\Delta C_{pu}$  is a change in heat capacity upon protein unfolding in the absence of ligand;

$[L_{T_m}]$  is a free ligand concentration at  $T_m$ ; and

$R$  is the gas constant.

60. (Previously Submitted) The method according to claim 59, further comprising the step of:

(6) inserting actual values  $\Delta H_u^{T_0}$  and  $\Delta C_{pu}$  into the equation of step (5).

5 61. The method according to claim 59, further comprising the step of:  
step (5).

(6) estimating values for  $\Delta H_u^{T_0}$  and  $\Delta C_{pu}$  for use in the equation of

10 62. The method according to claim 61, wherein step (6) comprises the steps of:

(a) fitting the data with a least squares algorithm according to: -

$$y(T) = y_u + \frac{(y_f - y_u)}{1 + \exp \left[ \left[ \frac{-\Delta H_u}{R} \right] \left[ \frac{1}{T} - \frac{1}{T_m} \right] + \left[ \frac{\Delta C_{pu}}{R} \right] \left[ \left( \frac{T_m}{T} - 1 \right) + \ln \frac{T}{T_m} \right] \right]}$$

where,

5  $y(T)$  is fluorescence unfolding data as a function of temperature  $T$ ;

$y_u$  is a pre-transitional fluorescence intensity,

$y_f$  is a post-transitional fluorescence intensity;

$\Delta H_u$  is a protein unfolding enthalpy;

$\Delta C_{pu}$  is a change in heat capacity upon protein unfolding;

10  $T_m$  is a midpoint temperature for protein unfolding transition in the presence of the ligand; curve fit

(b) varying values for ,  $y_u$  ,  $y_f$ ,  $\Delta H_u$ ,  $\Delta C_{pu}$ , and  $\Delta C_{pu}$  until a suitable

is obtained; and

(c) using resultant values from step 9(b) in step (5).

15 63. (Previously Submitted) The method according to claim 59, further comprising the step of:

(6) determining a ligand binding constant, for any temperature  $T$ , according to:

$$K_L^T = K_L^{T_m} \exp \left\{ - \frac{\Delta H_L^T}{R} \left[ \frac{1}{T} - \frac{1}{T_m} \right] + \frac{\Delta C_{pL}}{R} \left[ \ln \left( \frac{T}{T_m} \right) - \frac{T}{T_m} + 1 \right] \right\}.$$

64. (Currently Amended) A method for identifying a ligand that binds to a protein,  
20 comprising the step of:

(1) receiving unfolding data that indicates thermal unfolding as a function of temperature for a protein incubated with a molecule tested for binding;

(2) determining an unfolding temperature midpoint for the protein in the presence of the molecule from the unfolding data;

(3) comparing the unfolding temperature midpoint for the protein incubated with the ligand with the unfolding temperature midpoint for the protein in the absence of any molecules tested for binding; and

(4) determining that the molecule tested for binding binds to the protein when a difference between the unfolding temperature midpoint for the protein in the presence of the molecule and unfolding temperature midpoint for the protein in the absence of any molecules tested for binding exceeds a threshold; and

(5) estimating the ligand binding affinity at the unfolding temperature midpoint; and

(6) determining a ligand binding constant, for any temperature T, according to:

$$K_L^T = K_L^{T_m} \exp \left\{ - \frac{\Delta H_L^T}{R} \left[ \frac{1}{T} - \frac{1}{T_m} \right] \right\}$$

66. The method according to claim 63, wherein step (1) further comprises the step of plotting thermal unfolding according to a non-linear, least square fitting algorithm.

Claims 67-70. (Cancelled).